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ON THE NATURE AND FUNCTION OF "CHALKY" DEPOSITS IN THE SHELL OF OSTREA EDULIS LINNAEUS

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Introduction

Our textbooks of zoology, dealing with the anatomy of Lamellibranchia, usually state in describing the structure of the shell that each shell consists of three layers: a thin outer ''horny'' layer (periostracum) of purely organic nature (conchyolin); next a calcareous layer consisting of prisms placed vertically; and further a thinner or thicker inner nacreous layer, consisting of thin lamellae placed horizontally, and sometimes displaying a delicate lustre.

Many zoologists are not aware that this scheme, based on the anatomy of *Anodonta,* does not always hold good in other species of lamellibranchs. In fact the exceptions are far more numerous than the species which follow this scheme. A periostracum is not always conspicuous, layers of prisms are quite rare in the realm of the lamellibranchs, and only a few families possess a layer of real nacre (consisting of aragonite). Instead of nacre one often finds a sub-nacreous layer (made of calcite and without the peculiar lustre), or the so-called crossed-lamellar layer.

Not every investigator working with oysters has found time to study the shell structure of these animals in detail, or to read what others have already recorded about it. Thus we can easily understand that mistakes are often made when an author tries to force his findings into the textbook scheme.

The Structure of the Oyster Shell

In the shell of the oyster one can distinguish the following elements:

A. *The periostracum.* Generally the outer margin of the mantle of a lamellibranch mollusk is connected with the free edge of the shell by a sheet of conchyolin. During shell growth this sheet, the periostracum, is continually produced in the furrow between the outer and middle folds of the mantle edge. As this proceeds the mantle is enabled to protrude from the shells when held ajar. Then the outer fold of the mantle edge can start secreting layers of calcareous material on the outer sheet of the doubled periostracum, thus enlarging the shell. In this way the outermost layer of the shell necessarily comes to consist of the periostracum itself. Further, the ever-present periostracum insures an appropriate isolation of the shell-secreting outer fold of the mantle edge, thus preventing the calcareous deposits from being washed away while still in liquid form.

Curiously enough, the oyster at first sight does not appear to possess a periostracum, and its mantle edges appear to be capable of free movements to and from the shell's free edge, apparently not hampered by a trammeling periostracum. This misled many an investigator. Very few observed the oyster's periostracum, this being extremely thin and hyaline and, moreover, very elastic. It is easily overlooked but always present. Its elasticity leaves the mantle considerable freedom of motion and yet offers the required degree of isolation to the shell-secreting tissues. As far as I am aware, Leenhardt (1926) in his excellent pioneering study of the microscopic anatomy of the Portuguese oyster, *Gryphaea angulata*, was the first to demonstrate the presence of a true periostracum in oysters. Serial sections revealed its production in the outer furrow of the mantle edge. In his description Leenhardt (possibly under the influence of the treatise of Rawitz, 1888) made a minor mistake by supposing the wrong epithelium to be responsible for a thickening of this periostracum. I have been able to check in my own serial sections *(Ostrea edulis)* that it is the inner epithelium of the outermost fold of the mantle edge which does this thickening, and not the ciliated outer epithelium of the middle fold, as Leenhardt suggested. Ranson (1939, 1943) describes the periostracum of *Ostrea edulis* as it appears *in situ*, and points out its extreme tenuity (''épaisseur de l'ordre du millième de millimètre''). This led him to call it a pre-periostracum, as no sclerification into a ''true'' periostracum takes place in the oyster. Ranson's description can easily be checked by anyone disposed to observe a living oyster, carefully opened, and placed under water in adequate light. Ranson mentions that von Nathusius-Königsborn (1877) was the first to describe the oyster's periostracum, but I cannot make out from this investigator's paper that he really detected the extremely thin periostracum. There are no indications that von Nathusius-Königsborn ever studied a living oyster or made serial sections of its mantle edge, and only these two methods can lead to a demonstration of the periostracum. Therefore, I believe that Ranson himself was the first to describe a periostracum in *Ostrea edulis,* whereas Leenhardt demonstrated its presence in the Portuguese oyster several years earlier.

B. *Prismatic layer.* Curiously enough, the oyster shows a well-developed prismatic layer only on its right or flat valve. It consists of elastic brown scales placed in imbricate rows all over the outer surface of the flat valve and forms its extreme margin (about 5 to 10 mm. broad), thus contributing to the oyster's faculty of impervious closure. Though several authors (e.g., Orton and Amirthalingam, 1927), mistake this layer of scales for a periostracum, apparently misled by its "horny" appearance, it has repeatedly been stated that this is the oyster's true prismatic layer. Leeuwenhoeck (1682) was the first to detect the prisms, which are placed obliquely in the scales, and are of quite an irregular shape. Later Bowerbank (1844), Carpenter (1845), Rose (1859), and Tullberg (1881) stated that the scales of the oyster's flat valve represent a prismatic layer. Bøggild (1930) in his important work on the shell structure of mollusks, described the oyster's single prisms as curved and reclining in a characteristic manner. Schmidt (1931) studied the minute structure of the prisms and demonstrated that they do not consist of one single crystal of calcite but of a crystal-aggregate. Finally Douvillé (1936) tells us how he witnessed the formation of prisms during shell growth. Personally I repeatedly observed the deposition of the prisms on newly formed lamellae, a sight of great beauty when observed through a petrological microscope.

C. *Subnacreous layer (calcite-ostracum).* By far the greater part of the oyster's shell is constructed of foliated horizontally deposited layers of calcite, for this reason called the calcite-ostracum. It resembles nacre but lacks its beautiful iridescence. Therefore Carpenter (1845) called it the subnacreous layer. Later it was discovered that this subnacreous layer is constructed of fine lamellae of calcite, while true nacre always consists of aragonite-lamellae, arranged in a highly regular manner. Rose (1859) and von Nathusius-Königsborn (1877) state that the lamellae of the oyster's calcite-ostracum show a fine striation, the direction of which varies in alternate layers. Bøggild (1930) classifies the oyster among mollusks possessing shells of foliated structure and states that its separate folia are quite irregularly shaped, while their optical axes show a rather accidental position. As the majority of the folia are placed nearly horizontally, this gives rise to the characteristic pearly lustre of the oyster shell.

D. *Hypostracum.* This layer, described by von Nathusius-Königsborn (1877) and Tullberg (1881), is a rather thin layer in the calcite-ostracum from which it is distinguished by its somewhat different appearance. It is produced under the muscle and thus depicts in sections of oyster shells the course the muscle scar has taken during the life of the oyster. In the oldest parts of the shell this hypostracum is deeply buried under layers of calcite-ostracum subsequently deposited. Generally this hypostracum is somewhat more hyaline in appearance than the rest of the subnacreous layers. In oysters of the *Gryphaea* type, where the muscle scar is pigmented, the hypostracum is seen to traverse the shell layers as a colored band.

E. *Conchyolin sheets.* Sometimes we find thin layers of a greenish or brownish color imbedded in the calcite-ostracum. They are of different sizes and consist of organic material (conchyolin). Often one finds small single calcite rhombohedra imbedded in these layers (Rose 1859). They are secreted by the oyster either as a defense against the intrusion of boring worms *(Polydora ciliata)* or without any obvious reason. In some European oyster districts (e.g., locally in the Basin of Arcachon) such organic layers in the subnacreous material are quite common.

F. *"Chalky"* deposits. A conspicuous characteristic of the oyster shell is the local occurrence of opaque white masses of "chalky" appearance and of a soft texture which are imbedded in the harder translucent layers of the subnacreous material. We need not be surprised that many investigators described these "chalky" deposits and tried to explain their structure and function.

So far as I am aware, Gray (1833) was the first to record the occurrence of "calcareous particles deposited in a chalky or concretionary state between the proper laminae of shell structure." Later (1838) he added that the "chalky" matter is deposited in a succession of plates covered over with harder calcareous plates, more dense and crystalline in their construction. Carpenter (1845) corroborated Gray's observations and commented: "but I cannot regard such layers as forming part of the proper structure of the shell since the particles of carbonate of lime of which they consist, are not connected by any organic basis." Rose (1859) found small calcareous bars and grains in these layers, "die aber eine regelmässige Form nirgends erkennen lassen" and criticises the denomination "chalky deposits," as they have nothing to do with true chalk. Von Nathusius-Königsborn (1877) tried to study the minute structure of these deposits but found how difficult it is to produce satisfactory microscopic slides of this material. He persevered, however, and demonstrated that it consists of an irregular and complicated system of septa, predominantly placed vertically, and enclosing a multitude of small air-filled cavities: "Das ganze Gewebe stellt also ein in verscheidenen Richtungen sich kreuzendes System von zarten Stäbchen oder Fasern dar, die zwar wieder in verschiedenen Richtungen, aber doch in einer dominierenden — der senkrechten — durch Plättchen oder verkalkten Membrane verbunder sind.'' Clear figures elucidate his findings. Von Nathusius-Königsborn speaks repeatedly of air-filled enclosures, these certainly occur in dry shells in a museum collection, but he did not seem to be aware that under natural conditions these cavities are filled with sea water. Orton and Amirthalingam (1927) state that the ''chalky'' deposits, though apparently amorphous to the naked eye, appear to have a microcrystalline structure when examined through a petrological microscope. Bøggild (1930) states that it is a characteristic feature of many oyster shells that layers of normal consistency alternate with others more or less porous, which have all their elements placed vertically, while in sections parallel to the surface of the shell these folia are oriented in all possible directions *(Ostrea edulis).* Ranson (1941) studied intact fragments of these layers with a binocular microscope, and found ''très fines lamelles scintillantes, très longues, disposées perpendiculairement aux lames subnacrées limitantes, c'est-a-dire en principe verticalement''; ''lamelles empilées irrégulièrement les unes contre les autres''; ''elles sont constituées d'une fine membrane calcaire de soutien au sein de laquelle se trouvent de nombreuses fibrilles ou bâtonnets plus ou moins longs disposés longitudinalement et obliquement.'' Ranson's description reveals that the ''chalky'' deposits do not consist of an amorphous mass of calcite; on the contrary ''le calcaire est donc bien cristallisé au sein de ces lamelles.''

Chemical Composition and Some Physical Properties

We may wonder whether data on the chemical composition can give us any further insight into the true nature of the different layers of the oyster shell. Brandes and Bucholz (1817) estimated that the oyster shell consists of about 98.6% $CaCO_3$ and about 0.5% organic material. Chatin and Muntz (1895), analyzing likewise the entire shell found 86.5% to 95.9% $CaCO_3$, 0.71% to 0.99% $MgCO_3$, 1.3% to 1.7% $CaSO_4$ and 0.9% to 1.0% organic matter.

More interesting are the analyses of the separate layers taken from the oyster's shell. It was Schlossberger (1856) who procured such data:

Prismatic layer 89.1% $CaCO_3$ and 6.3% organic matter plus water.

Calcite-ostracum 94.7% to 98.2% $CaCO_3$ and 0.8% to 2.2% organic matter plus water.

''Chalky'' deposits 88.5% $CaCO_3$ and 4.7% organic matter plus water.

There are but few more recent analyses available. Bull (1927) estimated that ''chalky'' deposits consist of 78.5% $CaCO_3$ and 19.2% water plus organic material, and Douvillé (1936) mentions for the prismatic layer, 88.4% $CaCO_3$ and 4.8% albuminoids.

That the "chalky" deposits contain quite a lot of NaCl has been recorded by Rose (1859) and can be deduced from von Nathusius-Königsborn's statement (1877) that a solution made with the powdered "chalky" layers stirred in water gives a thick precipitate with $AgNO_3$.

Our knowledge of the structure of the "chalky" deposits, demonstrating its multitude of closed cavities filled with sea water, leads to the conclusion that there must be inevitably some sea salts inclosed in these layers.

The scantiness of data led me to collect pure samples of the different layers of the oyster shell (excluding the extremely thin periostracum) and to ask Dr. A. Grijns of the Bergen-op-Zoom Fisheries Laboratory to make an analysis of them, to which request he kindly acceded. After a thorough drying, a sample was weighed and dissolved in hydrochloric acid; next the calcium was precipitated as oxalate and estimated in the form of CaO (Treadwell, 1923, Vol. II, pp. 61-62). The percentage of nitrogen was estimated by the Kjeldahl method (modification Gunning Atterberg), from which figure the conchyolin content was calculated by multiplying with 6.9, since Schlossberger demonstrated that conchyolin from oyster shells contains 16.7% N. Another sample was extracted with water; in the solution thus obtained, chloride was titrated in the usual way and from that figure the quantity of sea salts has been computed. As I was especially interested in the relation between $CaCO_3$, conchyolin, and sea salts, I did not bother about the possible presence of some minor constituents. In a second set of samples, collected a year later, conchyolin only was checked to investigate whether the figures obtained in analyzing the first set were really representative in this respect. The results of these analyses are tabluated below:

	Prismatic-layer	*Calcite-ostracum*	*"Chalky" deposits*
$CaCO_3$	94.7%	98.5%	90.9%
Conchyolin	3.4%	0.6%	1.1%
Sea-salts	0.1%	0.1%	6.5%
Not recognized (silt, sand, etc.)	1.8%	0.8%	1.5%
	100.0%	100.0%	100.0%
Special conchyolin estimation in second set of samples	4.5%	0.5%	0.8%

Though not exactly the same, these figures correspond very well with the old data contributed by Schlossberger and with those of Douvillé. Those of Bull cannot be compared because we do not know the relation between water and organic matter in his samples.

What can we deduce from our figures? In the first place, we see that the prismatic layer—the scales of the flat valve—although apparently constructed of "horny" material is composed in reality of a very high percentage of $CaCO_3$ and only about 4% of organic matter. Knowing this, it surprises us that the scales of this layer are in practice so elastic that they have an important share in the oyster's impervious closure.

In the second place, parts of the calcite-ostracum free from "chalky" enclosures are composed of a very high percentage of $CaCO_3$ and only 0.5% to 0.6% of conchyolin.

In the third place, the "chalky" deposits, by some earlier investigators thought free of organic matter, contain more of it than the normal calcite-ostracum does. Since we now know more about its minute structure and since Ranson showed that its elements after decalcification show a dry residue of about 1.6%, it does not surprise us to find quite an amount of conchyolin in these deposits. At first sight it seems very odd that the "chalky" deposits contain the smallest percentage of calcium carbonate of all the layers of oyster shells, the "horny" prism-layer included. It would have surprised Gray to hear that, for he thought the "chalky" deposits to be virtually pure $CaCO_3$, without organic elements, and therefore did not want to reckon them among the "proper structure of the shell." We see, however, that it is the admixture with a considerable quantity of sea salts, which is the cause of the lower $CaCO_3$ content. Sea water is enclosed in the multitude of small cavities in the "chalky" deposits, and therefore we find it in our dried samples as salts. If we consider the "true" shell material, there is little difference in chemical composition of calcite-ostracum and its embedded "chalky" deposits, the latter showing a somewhat higher percentage of conchyolin.

As to some physical properties, we find in the literature the data of Bütschli (1908) who states that the specific gravity of the prismatic layers is 2.6 and of the calcite-ostracum 2.7. He worked with powdered shells, so was not aware of the porous nature of the "chalky" deposits. Ranson (1941, 1943) actually saw their porous nature, but only mentions that these layers contain a good deal of water.

I checked the specific gravity of intact parts of shell (after drying) and came to 2.5 for the calcite-ostracum (devoid of "chalky" material) and to only 0.5 for the "chalky" deposits. This indeed is a great difference! As some 6% of sea salts are imbedded in this air-dry material, the figure 0.5 is still too high if one wants to consider the quantity of calcite necessary for the construction of a given volume of shell material.

The Mechanism of the Deposition of "Chalky" Layers in the Oyster Shell

Some authors claim that they can advance a plausible explanation of the fact that the oyster deposits "chalky" layers in certain places in its shell and not in others. Pelseneer (1920) expresses himself quite cautiously in advocating a possible secondary solution of lime: "Ainsi, chez Ostrea, la couche moyenne est la moins riche; puis vient l'externe et enfin l'interne, de sorte, que tout se passe comme il s'était produit, apres le depot de calcaire un phenomène de redissolution et de réabosorption pour la sécrétion de la couche interne."

Anyone who has examined oyster shells during the growth season knows that "chalky" layers are deposited in a porous state and that they are covered with harder layers only later. Their porous structure cannot be attributed to a secondary solution of lime.

Orton and Amirthalingam (1927) suggest more positively that "chalky" deposits can be expected as soon as the oyster's mantle epithelium loses contact with the shell. It is assumed that the oyster secretes "chalky" material in order to restore the contact as quickly as possible. The most important layers of "chalky" deposits are to be found opposite the oyster's exhalent chamber, just beyond the scar of the adductor muscle, and this especially in the cupped valve. Orton states that the oysters usually are to be found on the beds with the cupped valve uppermost, and that in this position the epithelial ceiling of the exhalent chamber —not supported by visceral tissues—is inclined to sag, so that the contact between mantle and shell is lost here. Hence the secretion of "chalky" deposits especially in this particular part of the oyster's cupped valve.

Ranson (1939-1941) follows Orton in this reasoning without hesitation: "il s'agit tout simplement de décollements locaux du manteau, résultant de la présence de sillons ou autres cavités variées que le manteau ne peut poursuivre, par suit d'une modification des conditions de sécrétion de la bordure de la coquille."

I could not believe in this theory. It is a fact that "chalky" deposits are far more numerous opposite the exhalent chamber in the cupped valve than in the flat valve, in any case in oysters of about three to five years old. I doubted, however, whether this could be explained by the oysters lying flat valve undermost and by a subsequent sagging of the exhalent chamber's ceiling. I often examined oysters lying on the beds in the Oosterschelde (Holland). Oysters which are strewn on the beds from a moving boat—which is the usual procedure here—will reach the bottom with the cupped valve undermost, owing to their shape and the laws of friction. On a relatively soft bottom they will seldom be overturned afterward; on a hard and smooth bottom, on the contrary, strong currents can easily overturn oysters lying on the cupped valve, whereas oysters lying with the flat valve undermost are not easily affected by the current. Intermediate cases are found on bottoms of intermediate firmness. In such places I counted in fact about 50% of the oysters lying with the cupped valve undermost and about 50% lying on the flat valve (observations at extremely low water). Yet all our oysters show the bulk of "chalky" deposits in the cupped valve.

A simple experiment confirmed these field observations. In the spring of 1942 I placed about 25 medium sized oysters on a tray, cupped valve undermost, and covered them with another tray in such a way that the oysters could not be overturned. Repeated controls showed that the oysters remained lying cupped valve undermost. In a similar tray I placed about 25 oysters with the flat valve undermost. All these oysters showed excellent growth and have been analyzed late in 1942. Some of them showed "chalky" deposits recently produced beyond the muscle scar. Early in 1942 none of these oysters showed "chalky" deposits opposite the exhalent chamber as analysis of a control sample demonstrated.

Analysis of experimental oysters, November 1942:

25 oysters, cupped valve undermost during the growth season of 1942

24%	no "chalky" deposits	opposite the exhalent chamber in the cupped valve
44%	moderate quantities	
32%	much "chalk"	

23 oysters, flat valve undermost during the growth season of 1942

39%	no "chalky" deposits	opposite the exhalent chamber in the cupped valve
26%	moderate quantities	
35%	much "chalk"	

In my opinion the differences observed between the two series are quite insignificant. In each group I found only one oyster with "chalky" deposits in the flat valve opposite the exhalent chamber. We cannot understand how "chalky" layers are deposited in oysters lying cupped valve undermost if the "sagging of the ceiling theory" is assumed to hold good, and we cannot understand why these very same oysters do not produce "chalky" deposits in the flat valve as the topmost mantle epithelium of the exhalent chamber is certainly as liable to sagging in these oysters as it is supposed to be in oysters lying flat valve undermost. In fact my experimental oysters lying cupped valve undermost show even rather more "chalky" material than those of the other group. This leads me to reject the hypothesis of Orton and Amirthalingam and with it Ranson's reasoning.

In considering the mechanism of the deposition of "chalky" layers in the oyster shell, I believe we ought to confine ourselves to stating that obviously every part of the shell-secreting mantle tissue possesses the faculty of constructing both the harder layers of the calcite-ostracum and "chalky" deposits. This is often carried out alternately. Until we know more about the biochemical events which guide shell-secretion, we find ourselves forced to leave undecided how the mantle epithelium succeeds in depositing "as locally required" either subnacreous or "chalky" layers. Which factor can be held responsible in determining what kind of material is "required" will be discussed in the next section of this paper.

First I would like to draw attention to Ranson (1940, 1943) who advanced still another factor in explaining the mechanisms of deposition of "chalky" layers. Only in places where the substrate is rich in calcareous material can one expect oyster shells with ample "chalky" deposits, according to him, while such layers should be absent in oysters living in areas poor in lime: "La pauvreté en calcium des fonds où croissent les huîtres semble être ici le facteur déterminant de l'absence des couches crayeuses." In his book (1943) he states: "Dans le bassin d'Arcachon, dont le fond de sable est pauvre en calcaire, Ostrea edulis et Gryphaea angulata ont une coquille très mince, presque translucide." This surprises me very much. The Dutch oyster farmers imported oysters *(Ostrea edulis)* from the Basin of Arcachon more than once, and in 1947 I visited this area myself, on which occasion I saw quite a lot of oysters. The shells of these oysters seemed thicker than anywhere else and possessed ample "chalky" deposits.

To explain why the shells of the Brittany oysters contain so much "chalky" deposit though the underlying bedrock here consists of acid granite, Ranson tells us that so many shells have grown there for so many generations that the bottom layers of the Morbihan bays gradually became quite rich in calcareous material. Ranson does not explain why this is not the case in the Oosterschelde and in the Basin of Arcachon, reckoned by him among the areas poor in lime.

In my opinion, Ranson's main mistake is that he seems to ignore that the quantity of calcium present in sea water is practically the same in the different parts of the Atlantic coast, viz., about 1 gram of $CaSO_4$ per liter. Unlike fresh water, the calcium content of sea water does not depend on the nature of the subsoil but on ionic balances and solubilities.

Fox and Coe (1943) have shown for the mussel *(Mytilus californianus)* that it is absurd to assume that the lime necessary for shell deposition is derived from the food ingested, and that we should suppose that mollusks take their lime either directly from the water or ingest it in particulate inorganic form. The particulate form being the debris of older shells we wonder how those gained their lime in earlier times in "acid" areas if the particulate form is the only one which can be used. I believe that mollusks, corals, and so on can take the calcium they need directly from the sea water where it is present in solution in large quantities. I believe that it does not matter, even if calcium is present in sea water largely as

$CaSO_4$. Experiments with hens (Irvine and Woodhead 1888-89) have shown that $CaSO_4$, $CaCl_2$, and other calcium compounds are as good a source of calcium in the formation of egg-shells as $CaCO_3$, if administered in the fowl's food. Further it is only arbitrarily concluded (from the sequence of events in evaporation) that the calcium is present in solution in sea water in the form of $CaSO_4$. Is it not better to express the calcium present as the number of calcium ions in sea water? Nobody can prove that it is not partly $CaCl_2$ mixed with Na_2SO_4 instead of $CaSO_4$ and NaCl. Living creatures possess the remarkable faculty of concentrating a variety of elements within their own bodies or shells against a concentration gradient. There is no reason to believe that oysters and other lime-secreting animals do not possess the power to "catch" in some still unknown biochemical way the calcium ions they need from the abundance in which they are permanently bathed.

THE POSSIBLE FUNCTION OF "CHALKY" DEPOSITS IN THE OYSTER SHELL

Though in the foregoing section I criticized certain opinions expressed by Orton and Ranson, I fully agree with other points in their papers. Orton and Amirthalingam (1927) state: "In very young oysters (1 to 2 years old) chalky deposits occur fairly frequently and irregularly, but obviously in places where a thick layer of shell material is required to fill up a space." Further: "It is submitted that the function of these deposits is to fill in rapidly depressions under the mantle or secreting epithelium, which depressions cannot be maintained in the physiological state of the oyster in that instant, or which can only be maintained with loss of its efficiency in functioning." And: "All chalky deposits of shell material are due, on the whole, to local unsuitabilities of the contours of the shell to the need of the oyster." Orton (1937) added that "chalky" deposits "are rapidly made to smooth out the inner contour of the shell. Such deposits are copious in regularly growing brood of *Ostrea edulis,* and particularly in the irregularly shaped brood grown on twigs in the Norwegian oyster polls."

Ranson (1939-1941) tells us: "couches crayeuses de peu d'importance remplissent des sillons du bord de la coquille," and further: "le resultant suggerait: l'animal avait besoin de remplir rapidement une cavité devenue inutile et qui le gêne," though I should add here, that Ranson himself shrinks from this reasoning and immediately resorts to the hypothesis of dislocation of mantle and shell to find an appropriate causal relation.

I entirely agree with the view that the oyster makes use of "chalky" deposits to smooth out the inner contours of its shell. Irregularly shaped oysters, e.g., those attached in crevices, growing on stones, or growing in clusters tightly together, succeed in creating a normal smooth shell interior by producing ample "chalky" deposits. Further, I have often observed that the extreme edges of the shells of young oysters growing well in a place not too exposed to the surf are inclined to take a somewhat undulating shape. As shell growth proceeds, the oyster always smooths out the inner contours of the shell by filling up all the little furrows with "chalky" material. This procedure certainly is very economical. For from my estimation of the specific gravity mentioned above, it follows that the oyster thus can fill up a space with less than one-fifth of the shell material (both organic and inorganic) that would be required if the normal harder layers were to be deposited. With good reason we can state that the oyster uses these "chalky" deposits as a "cheap padding."

The fact remains, however, that in oysters of three years and older, by far the most important "chalky" deposits are to be found opposite the exhalent chamber, just beyond the muscle scar, in a place where we cannot detect a space which requires filling in to smooth out the shell's interior contours. The older the oyster, the more successive layers of "chalky" material appear to be deposited there.

This fact has been recognized by virtually all the workers in this field. Gray (1838) has already recorded that those layers are found "commonly forming a convex spot in the cavity of the oyster, just beyond the scar of the central adductor muscle." Others corroborate this finding. When we follow the events in a growing oyster, we see that deposition of "chalky" layers beyond the muscle scar begins at an age of about three years, and then in the cupped valve only. In due time the deposited porous layer is covered by harder layers of the normal subnacreaus type. Later the entire shell puts on a new rim of growth, the muscle moves away from the hinge—as it has been doing ever since the oyster started growing—and a new layer of chalky material is deposited beyond the muscle scar opposite the exhalent chamber. In due time a harder subnacreous layer is found to cover this second porous layer, too. This goes on as depicted in figure 1. Every succeeding "chalky" layer is placed somewhat farther away from the hinge than its predecessor owing to the steady displacement of the muscle. Beginning with the oyster's fifth or sixth year, "chalky" layers are deposited not only in the cupped valve but also in the flat valve, here too, opposite the exhalent chamber. The older the oyster, the more deposits we find till at last in some very old and thick oysters, it is difficult to tell at first sight which is the cupped and which the flat valve. The oysters depicted in Petersen's paper (1918), though not accompanied by an explanation of this kind, can assist us likewise in following events during the oyster's development. Tullberg (1881), however, was led astray and depicted in his paper a section of an old oyster in which there is no difference whatever between cupped and flat valve. A closer observation reveals that he depicted two cupped valves connected together, which is not correct. A photograph is more trustworthy in this field, and the very old oyster, shown in figure 2*, clearly shows the actual relations and the permanent difference between cupped and flat valve. The older parts of the shell demonstrate especially clearly that the deposition of "chalky" layers is far more important in the cupped valve even when the "flat" valve is inclined to take a "cupped" shape as can be observed in some, but not in all, old oysters (cf. figure 1). Moreover, only the flat valve possesses a scaly prismatic layer, as has been discussed above, but this layer may be easily lost on dry specimens.

What is behind this rhythmic deposition of "chalky" layers in this particular part of the oyster's shell where we see no obvious need to fill in an inconvenient space?

Southern (1918), who stated "at the approach of the succeeding spawning season the gonadal cavity is again enlarged by the rapid deposition of soft shell substance in that part of the shell which surrounds the gonadal cavity," was obviously wrong, as only the addition of a new rim to the entire shell can enlarge its cavity; the more the angle of this rim deviates from the horizontal, the deeper the shell becomes.

Bjerkan (1918) believed, from studying the oyster depicted in figure 2, that the thin dark layers seen between the "chalky" deposits, considered by him to be layers of conchyolin, are produced in summer, whereas the thick "chalky" deposits are laid down in the winter season: "De tynde konchyolinlamellar som gaar fra laasen og ut mot skallets rand avsættes om sommeren, en ny lammelle for hver sommer. Særlig om vinteren avsættes der imidlertid kulsur kalk mellem lamellerne." Bjerkan believed that counting the number of "chalky" layers in sections of oyster shells is a very reliable method of determinnig the oyster's age. Even if we assume with Bjerkan that one "chalky" layer is produced each year (and never two or none, which remains to be demonstrated), we should remember that deposition of "chalky" layers beyond the muscle scar only starts at an age of 3 to 4 years, and not at the same age in different oysters and in different oyster districts. It certainly is not a weathering off of older shell-parts which is responsible for the distance between the hinge and the place where "chalky" deposits begin, as Bjerkan believed: "Den store avstand mellem skjællets laas og den første lamelle lar imidlertid til at antyde at et ældre parti av skallet er forvitret." This phenomenon is no doubt due to the advanced age at which the deposition of these layers begins. If we cannot agree with Bjerkan's explanation of this de-

* I am indebted to Dr. P. Bjerkan, Bergen, Norway, who kindly sent me a copy of this photograph, upon my request.

tail, we must admit that his method is still the best to esitmate the age of an old oyster. Also that it is quite reliable if oysters from one and the same region are considered and if oysters of an accurately known age which are grown in the same region are available for comparison in order to check the age at which deposition of "chalky" layers starts.

Douvillé (1936) believed that soft material is deposited in the months without an "R" and harder layers in those with an "R" (just the oppo-

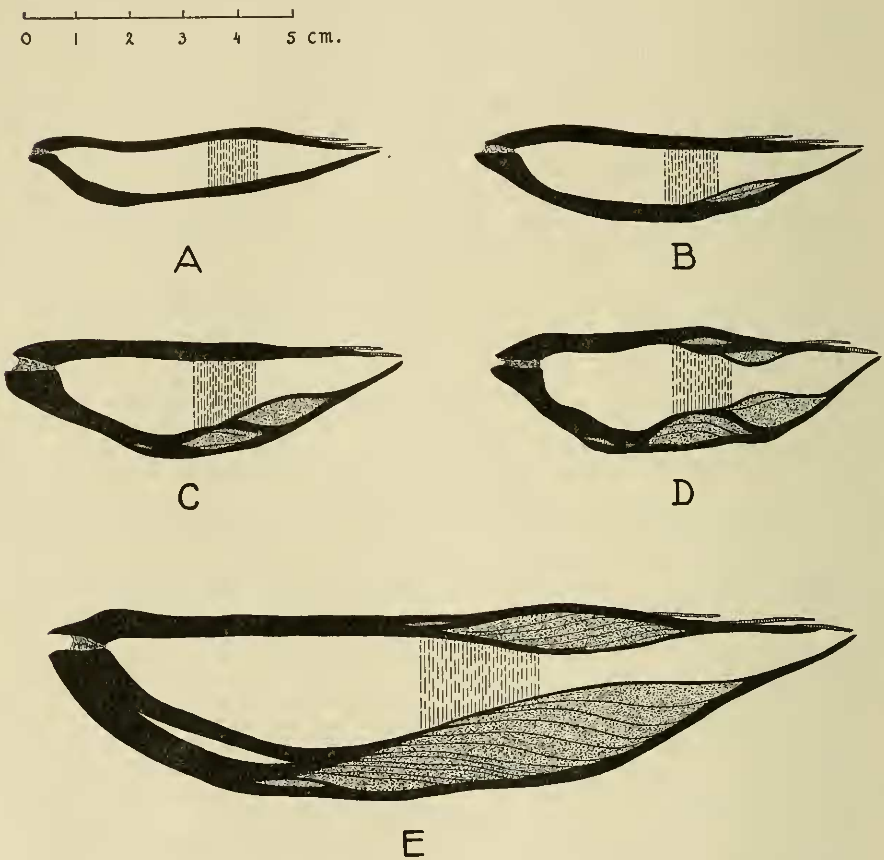

Figure 1. Cross-sections of oyster shells. Place and size of the adductor muscle is indicated. Filled in black: hard sub-nacreous layers. Hatched: prismatic layer. Dotted: "Chalky" deposits. Note the increasing depth of the part of the shell lodging the "body" of the oyster, while the distance between the valves is maintained at a constant level opposite the exhalent chamber (beyond the muscle). A, B, two Dutch native oysters. C, a relaid Brittany oyster. D, a relaid Arcachon oyster. E, a very old oyster from a natural bed in the North Sea, showing a chamber in the cupped valve.

site of what Bjerkan claimed). There is something in this view, as one finds the porous layer at the surface, newly deposited, during the period in which rapid growth occurs (which is the summer season here). By contrast, there is a covering of harder layers produced during the periods in which the oysters thicken their shells. These periods occur here during spring and autumn. Douvillé cannot explain, however, why the layers are deposited only in this particular place, and why not all over the shell's interior.

Orton's hypothesis of the sagging of the exhalent chamber's roof has already been discussed and rejected. Basing my argument on another part of Orton's reasoning I offer the following explanation:

In discussing the filling up of depressions, Orton states that these "cannot be maintained in the physiological state of the oyster in that instant or which can only be maintained with loss of its efficiency in functioning."

I presume that the oyster deposits "chalky" layers opposite the exhalent chamber, when growing older, *in its efforts to maintain its "efficiency in functioning."* That "chalky" material instead of harder layers is used indicates only the oyster's "economy"; *where possible the oyster always uses soft porous deposits when quite a lot of shell volume has to be produced, since this requires only one-fifth of the material which the deposition of harder layers demands.*

What is the result of the deposition of "chalky" layers beyond the muscle scar, in the cupped valve first, in both of the valves in older oysters? The result is that *the distance between cupped and flat valve always remains limited in this area, no matter how large and deep the part of the shells lodging the visceral and reproductive tissues of the oyster may become.* In oysters of three to four years and older which I investigated, this particular distance never differed much from 0.5 to 0.6 cm. This is clearly demonstrated by the oysters of figure 1, drawn accurately in natural proportions. The figure 0.5 to 0.6 cm. holds good for the degree of closure depicted. During violent contractions of the adductor muscle it will be somewhat lower; during feeding, on the other hand, while the shells are held slightly ajar, it will be somewhat higher.

I feel sure the oyster requires for its efficiency in functioning (perhaps in producing its regular flow of water, bringing food and oxygen) a very moderate height of the exhalent chamber. If the distance between the two valves is least opposite the exhalent chamber, there is only a slightly greater distance between the valves opposite the gills. The more the oyster is cupped, the more the size of the subsequent "chalky" deposits expands in a horizontal direction. In old oysters the "chalky" deposits laid down at regular intervals extend over a considerable part of the shell, opposite exhalent and inhalent chamber, but never under the oyster's visceral parts (i.e., under the true

Figure 2. Cross-section of a very old oyster, originating from the Norwegian coast. Bjerkan estimates its age at approximately 26 years. Note the convex shape of the "flat" valve, compared with that shown in figure 1, E. Chambers in both of the valves. The hypostracum, marking the course the muscle scar has taken, clearly visible.—Photo, Lea.

"body" of the oyster). What evidence can be adduced to corroborate my view?

In the first place, the figures in the paper of Petersen (1918) and in the booklet of Gaarder and Bjerkan (1934) and also the photograph depicting the large oyster (fig. 2) which has already been published by Bjerkan (1918), all show very clearly how shallow the region of the exhalent chamber remains even in old and deep oysters, which agrees with my oysters depicted in figure 1.

In the second place may be cited the results of a closer examination of oyster shells originating from different localities. If my theory is correct we can expect thicker or thinner layers of "chalky" material, according as the shells are more or less cupped in shape irrespective of the place where the oysters are grown.

To this end I compared the shells of the marketable oysters in my collections. These shells, all of a diameter of approximately 7 cm., were taken at random from groups of oysters four to five years old. I computed an index for the degree to which the oysters are "cupped" in the following way, using the cupped valve only, as the flat valve is still very flat at this age:

$$\frac{\text{height}}{\text{mean diameter}} \text{x } 100$$

I used as mean diameter the average of the greatest dimensions of the shell in two directions (parallel to the hinge and at right angles to it). To check the height I laid the cupped valve flat on the table and measured how much the highest part of the shell reached above the surface of the table. I am aware that for this purpose it would have been more accurate perhaps to measure the shell's interior, using a kind of conchograph like that devised by Vlès (1904), but my method required no intricate apparatus and gave quite a good idea of the "cuppedness" of the shells which were of a regular shape. The presence of "chalky" deposits beyond the muscle scar was estimated approximately by separating them into categories: "absent," "little," "moderate," "much," and "very much." The results of this investigation are tabulated below:

1. Dutch oysters, spat settled on tile-collectors; during second year grown in wire-covered trays. Excellent growth. Analyzed in their second winter. Mean diameter 6 cm.; 48 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	15	20.0
Little	10	22.0
Moderate	23	25.0
Much	0	—
Very much	0	—

2. Dutch oysters, spat settled on tile-collectors; grown on wire-covered trays in both 2nd and 3rd summer. Analysis 3rd winter. Mean diameter 7 cm.; 48 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	6	18.2
Little	9	21.3
Moderate	20	22.5
Much	13	23.2
Very much	0	—

3. Dutch oysters, spat settled on tile-collectors. After detachment, at 9 months, scattered on a deeper oyster bed (peat soil), and grown there during their 2nd, 3rd, and 4th summer. Analysis 4th winter. Mean diameter 7 cm.; 48 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	6	20.5
Little	12	22.8
Moderate	16	24.1
Much	13	27.0
Very much	1	30.0

4. Dutch oysters, spat settled on shell-collectors; grown on deeper parcels since. About 4 years old. Mean diameter 7 cm.; 35 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	4	21.8
Little	10	22.5
Moderate	13	24.4
Much	7	28.6
Very much	1	32.0

5. Brittany oysters, imported spring 1937, relaid in Dutch waters for one summer season. Analysis November, 1937. Mean diameter 7 cm.; 82 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	2	19.5
Little	14	22.7
Moderate	34	24.6
Much	27	27.3
Very much	5	28.2

6. Arcachon oysters, imported spring 1937; relaid in Dutch waters for one summer season only. Mean diameter 7 cm.; 51 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	1	23
Little	2	23
Moderate	21	26
Much	15	27
Very much	12	32

7. Brittany oysters, directly imported as full grown marketable oysters. Mean diameter 7 cm.; 36 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	0	—
Little	5	20.8
Moderate	10	24.2
Much	13	26.0
Very much	8	29.0

8. Arcachon oysters, imported as full grown marketable oysters. Mean diameter 7 cm.; 13 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	0	—
Little	0	—
Moderate	1	27
Much	4	28.5
Very much	8	30.2

9. French oysters from a natural oyster bed, Finistère. Mean diameter about 7 cm.; 23 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	0	—
Little	1	19
Moderate	5	25
Much	8	27.6
Very much	9	32.0

10. Italian oysters, imported as full grown marketable oysters. Mean diameter about 7 cm.; 9 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	0	—
Little	0	—
Moderate	1	26
Much	1	31
Very much	7	29

11. Norwegian oysters, imported spring 1937; grown in Dutch waters for one summer season. Mean diameter about 7 cm.; 12 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	2	21.5
Little	2	24.5
Moderate	5	25.8
Much	2	25.5
Very much	1	28.0

12. English oysters from the Truro beds, not yet ripe for the market. These oysters diverge somewhat from the round shape and approach a triangular shape. Mean diameter 6 cm.; 22 specimens.

"Chalky" deposits	*Number*	*Index*
Absent	3	23.3
Little	6	25.2
Moderate	10	25.6
Much	3	27.4
Very much	0	—

These figures clearly show that the presence of copious "chalky" deposits in the cupped valve of oysters of normal marketable dimensions (about 7 cm.) is not confined to oysters grown on beds characterized by a high percentage of calcareous material as Ranson suggested. Oysters showing "chalky" deposits beyond the muscle scar may be found among samples from practically any provenance irrespective of the culture method used.

Further, these figures indicate that there is a close correspondence between the quantity of "chalky" material deposited beyond the muscle scar and the degree to which the shell is cupped. I am aware that there exist individual variations, but if one works with not too limited numbers there is a marked correlation between the two figures. The deeper the shells are, the more "chalky" material we can expect to find. The shells ranked among those showing very much "chalky" material possess as a rule such deposits in the flat valve also.

Oysters of different provenance often differ in the percentage of shells showing copious "chalky" deposits. Our oyster farmers are correct when they say that relaid French oysters show more "chalk" in the shell than do native Dutch oysters. This corresponds with a greater degree of "cuppedness" of the French oysters. Oysters from Arcachon possess very deep shells and proportionally profuse "chalky" deposits beyond the muscle scar. If we compare Dutch oysters with French oysters and relaid French oysters, we find that the different categories of "chalky" deposits, distinguished as in the tabulation above, are to be found at nearly the same value for the index of cuppedness:

"Chalky" deposits	*Index (Dutch)*	*Index (French)*	*Index (relaid French)*
Absent	21.2	—	21.2
Little	22.7	20.8	22.8
Moderate	24.2	24.2	25.3
Much	27.8	27.2	27.2
Very much	31	29.6	30.1

The presence of calcareous material in the bottom deposits apparently has nothing to do with the formation of "chalky" deposits. Still there exists some kind of influence of environmental factors. Both hereditary qualities and environmental conditions (in the latter probably mainly the physical, possibly also the chemical and biological characteristics of the substrata on which the oysters are grown, and also the effect of currents and surf) determine whether the oysters are predominantly inclined to grow a cupped or a relatively flat type of shell, and with that the quantity of "chalky" deposits to be laid down in the shell.

In the third place, I tried to determine the significance of the narrow shell cavity beyond the muscle scar and to find out what happens when the "chalky" layers there are taken away. I anaesthetized some oysters and carefully took away a great deal of the "chalky" deposits, then placed them back on a tray. Though oysters can easily stand anaesthetization (with the aid of $MgSO_4$), the oysters bereft of their "chalky" deposits died, all of them within two months. Probably I treated them too roughly. I cannot tell what was the exact cause of death. Could it be that nutrition or respiration was impaired by interfering with the correct functioning of the gill-apparatus? Further investigations in this field, carried out with more delicate methods than I used, are required to find the answer to this question.

FORMATION OF CHAMBERS

I do not want to conclude this treatise dealing with features of the oyster shell without mentioning the phenomenon of "chambering." In many oyster shells we can detect shallow cavities, filled with sea water (often putrefied through anaerobic conditions), occurring predominantly in the cupped valve under the visceral parts of the oyster. Sometimes we find only one "chamber," sometimes an entire series of them. When the walls of these chambers are thin and easily broken by the knife in opening the oysters (which sets free the often evil-smelling contents of the chamber), the Dutch oyster farmers characteristically speak of "cat-ice" oysters. Such oysters are but rarely found in the Oosterschelde, the Dutch oyster center, although fairly often in several of the French oyster districts. Very old oysters almost always show a series of chambers in the cupped valve, in very old specimens even in the flat valve.

These chambers have been noticed by several investigators. Houlbert and Galaine (1916) discuss the occurrence of chambers in the oyster shell and state: "les couches de nacre successives ne sont point au contact, elles se sont formées à une petite distance les unes des autres, comme si l'animal, diminuant d'épaisseur, et habitant, par suite, une maison trop grande, avait voulu ramener sa demeure à ses propres dimensions."

Orton (1937) expresses the same view: "chambering appears to be due entirely to shrinkage of the body with subsequent automatic secretion of nacreous material by the surface of the shrunken body, destined normally for thickening the shell.'' Orton adds that a rapid rise in salinity brings about considerable shrinkage of the oyster's body and he holds this fact responsible for the formation of chambers in the oyster shells, together with the effect of ample spawning, which view had been expressed earlier by Orton and Worsnop (1923).

I entirely agree with these views. ''Chambrage'' is to be expected in oysters exposed to salinity changes so characteristic of many of the French oyster regions. In the Oosterschelde, where the salinity is remarkably equable, chambering seldom or never occurs in oysters three to five years old. Salinity changes are not the only factor to be held responsible for ''chambrage,'' since very old oysters living in the North Sea beyond the reach of marked salinity changes invariably show chambering of the shell. Possibly shrinkage owing to spawning is the cause of this phenomenon. In younger oysters spawning does not bring about chambering, in any case not in the rich Dutch waters where the oysters rapidly make up losses due to spawning. That the oyster *(Ostrea edulis)* is not able to counteract quickly the influence of a changing salinity is demonstrated by oysters which are suddenly exposed to reduced salinities. The living tissues soon look puffed up, a fact which is sometimes made use of by unscrupulous oyster dealers, in their efforts to sell lean oysters.

It is noteworthy that typical museum biologists once held different views concerning the chambers. Gray (1833) said, in dealing with oysters displaying a series of chambers, that there was no siphon passing from one septum to the other, thus indicating that it reminded him of the shell of *Nautilus* with its many chambers connected by a siphon passing through the septa.

Laurent (1839, 1839a, 1844) thought that he detected the siphon and really suggested that some kind of family relation might exist between *Nautilus* and *Ostrea!* What he thought to be a siphon, comparable to that of *Nautilus,* was the rudiment of the retractor pedis muscle, which connects the shell with the mantle tissues just opposite the labial palps. This muscle is seldom mentioned in the oyster literature. Elsey (1935) records its presence in *Ostrea lurida* and *Ostrea gigas.* I can state that this reduced foot retractor muscle is not of much importance to the oyster. In a number of oysters I cut these muscles on both sides, after anaesthetization. These oysters thrived normally afterward, not showing any signs of distress. Leenhardt (1924) assumes that they may play a part in keeping the animal in its proper place within the shell. This may be true, for some of my experimental oysters showed a partly doubled ligament, demonstrating a slight displacement of the epithelium which is responsible for the secretion of new layers of ligament.

It is now generally accepted that chambering is caused by shrinking of the oyster's tissues, which shrinkage is important where the meat of the oyster is thickest, i.e., in the true "body" of the oyster. Subsequently, deposition of shell layers, preceded by a thin sheet of conchyolin, takes place to adjust the size of the shell cavity to the size of the body. We, therefore, always find the chambers opposite the visceral parts of the oysters. Here is a true case of dislocation between shell and mantle. This apparently always results in the formation of a water-filled chamber and never in the deposition of "chalky" deposits as Orton and Ranson supposed.

It is easy to prove this experimentally. If an angular object is fixed in the shell's interior so that the mantle cannot easily reach the chinks, and if such oysters are kept under natural conditions, we will observe sooner or later that the object and its immediate surroundings are first covered with a sheet of conchyolin on which harder subnacreous layers are deposited in due time. Small chambers are thus created around the foreign object.

Summary

"Chalky" deposits in oyster shells consist of the same material as the harder subnacreous layers. They differ in that the opaque "chalky" deposits are of a highly porous structure, whereas subnacreous material is more solidly built. In depositing a given volume of "chalky" material the oyster needs only one-fifth of the shell-substance (both organic and inorganic) it would need if constructing it of subnacreous material. "Chalky" material is used by the oyster as a measure of economy, as a "cheap padding" in smoothing out the shell's interior and in creating the right shell shape to maintain its efficiency of function. This means that the oyster always keeps the distance between the two valves very limited where the exhalent chamber is located. In a lesser degree this also is true of the inhalent chamber. The more the oyster shell attains a cupped shape, the more layers of "chalky" material are deposited beyond the muscle to maintain the proper shell dimensions, which are apparently necessary for its well being.

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